

REVIEW ARTICLE

LIQUID BIOPSY AS A NON-INVASIVE APPROACH FOR CANCER DETECTION AND CLINICAL MANAGEMENT

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Abstract:

Cancer is one of the most serious health challenges worldwide and early diagnosis, accurate monitoring and personalized treatment are essential to improve patient survival. Traditional tissue biopsies have been the standard method for providing crucial information about the nature of tumors, but it is invasive, risky and has limited ability to reflect tumor heterogeneity or evolution. To overcome these limitations, liquid biopsy emerged as a powerful alternative which represents a safe and advanced non-invasive approach that enables the analysis of tumor derived materials from body fluids such as blood, cerebrospinal fluid, urine and saliva. Circulating biomarkers such as ctDNA, CTCs, EVs, cfRNA, and TEPs serve as valuable indicators of tumor dynamics and offer insights into the molecular landscape of cancer, tumor heterogeneity and dynamic changes during treatment. Advances in molecular technologies such as next-generation sequencing, digital PCR, and methylation-based assays can now identify even tiny molecular changes, including DNA methylation, which is proving useful for early cancer detection. It also helps in tracking treatment response and identifying cancer recurrence earlier than traditional methods and also have significantly enhanced the sensitivity and utility of liquid biopsy. It also highlights the advanced technologies that make this possible, like next-generation sequencing, digital PCR, and DNA methylation analysis. Beyond diagnosis, liquid biopsy plays an expanding role in tracking how patients respond to treatment, spotting cancer recurrence early, and helping tailor therapy to each individual. While there are still challenges to overcome, such as standardizing tests and detecting very early-stage disease, liquid biopsy is shaping a more personalized and less invasive future for cancer care.

Keywords: Cancer, Biomarkers, Liquid Biopsy, Diagnostics, Early Detection.

1. Introduction

World's one of the most leading causes of mortality is cancer as early detection and monitoring of disease remains critical for enhancing patient results. Cancer is a complex and life-threatening disease characterized by the uncontrolled growth and spread of abnormal cells in the body. The nearby tissues are invaded by these abnormal cells and in some cases these cells may also spread to nearby organs through the blood or lymphatic system through a process called metastasis. Many factors may contribute to the development of cancer in almost any part of the body such as genetic mutations, lifestyle, environmental factors and other conditions. For initial diagnosis and histopathological evaluation, traditional tissue biopsies were used to comprehend the characteristics of a tumor but they pose various limitations: they're invasive, sometimes risky, and often fail to capture the complete picture of a tumor's complexity, especially when the cancer has spread or evolved. To overcome these limitations, a new promising approach called Liquid biopsy has emerged as a non-invasive approach that allows to understand everything about a tumor and its components, such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), microRNAs (miRNAs), and extracellular vesicles (EVs) by just analyzing blood or from body fluids including blood, urine, and saliva. This advanced approach makes it easy to examine patients more often and beyond a simple overview, this perspective reveals the constantly changing and adapting nature of cancer.

As Molecular technologies are emerging and recent technological advancements such as Cancer Personalized Profiling by deep Sequencing (CAPP-Seq), digital droplet PCR (ddPCR), and nanopore sequencing have significantly enhanced the sensitivity and specificity of liquid biopsy, making it a valuable tool in modern oncology (Kato *et al.*, 2021; Si *et al.*, 2024). The use of the advanced techniques have the ability to identify mutations, epigenetic changes, and other alterations in various cancers which include lung, breast, colorectal, pancreatic, and gynaecological cancers (Fujii *et al.*, 2022; Overs *et al.*, 2024). Liquid biopsy offers a significant advantage in its capacity to monitor therapeutic response in real time, enabling clinicians to observe tumor dynamics throughout the course of treatment. It also plays a critical role in the early detection of cancer recurrence and in identifying minimal residual disease (MRD), areas where conventional tissue biopsies often fall short due to their inability to reflect the ongoing, dynamic changes within the tumor environment (Ben Sassi *et al.*, 2025; Hosoya *et al.*, 2022).

The scope of liquid biopsy has been expanded in recent years beyond genomic profiling. For the detection of cancer at an early stage, Epigenetic markers most prominently DNA methylation are gaining attention. Research has shown that specific methylation patterns in certain genes can act as dependable biomarkers for cancers such as colorectal, pancreatic, and those affecting the oral cavity (Misawa *et al.*, 2020; Overs *et al.*, 2024). Alongside this, transcriptomic and proteomic data retrieved from liquid biopsy samples are also being studied for their usefulness in refining cancer classification and predicting disease progression (Ding *et al.*, 2024; Moisoiu *et al.*, 2022).

That said, the path to routine clinical use is still developing. There are several hurdles that need to be addressed, ranging from the lack of standardization across testing protocols to limitations in detecting early-stage cancers, as well as the technical complexity of integrating diverse molecular data. This review explains the overview of liquid biopsy and its evolving role in cancer diagnostics and

treatment. It explains about the key circulating biomarkers, the technologies used to detect them, and their clinical significance across different cancer types. It also highlights recent advances in biomarker discovery and considers how liquid biopsy may continue to shape the future of personalized cancer care.

2. Types of Biomarkers in Liquid Biopsy

As the cancer diagnostics continue to grow there is a growing interest for minimal invasive methods of detection and diagnostics as it gives detailed information about tumors. By analysing biomarkers in the body fluids like urine, blood and saliva, liquid biopsy is emerging as a promising tool that allows real time analysis of tumors. The important biomarkers that help in liquid biopsy include circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), extracellular vesicles such as exosomes, cell-free RNA (cfRNA) including microRNAs (miRNAs), and tumor-educated platelets (TEPs). These biomarkers help in the early detection of cancer as well as help in prognosis and evaluate how a particular individual responds to treatments and also help in monitoring cancer treatments.

2.1. Circulating Tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) consists of tiny fragments of DNA released into the bloodstream by tumor cells, usually as a result of cell death through processes like apoptosis or necrosis. These fragments carry the same genetic and epigenetic makeup of the tumor itself, making ctDNA an incredibly useful marker for understanding the biology of cancer without the need for invasive procedures. ctDNA is also used to detect mutations as they guide targeted treatments and also monitor how a patient is responding to the therapy. In non-small cell lung cancer (NSCLC), for example, ctDNA is often tested for resistance mutations such as EGFR T790M that can impact what therapy is given next. In head and neck squamous cell carcinoma, rising levels of ctDNA have been linked to greater tumor burden and a higher risk of recurrence providing an early warning signal for relapse (Haring *et al.*, 2021). Beyond genetic changes, ctDNA also carries epigenetic information, such as methylation patterns, which has opened up new opportunities for early detection and risk assessment in cancers like oral, pancreatic, and colorectal (Misawa *et al.*, 2020; Budhathoki *et al.*, 2025; Overs *et al.*, 2024).

2.2. Circulating Tumor Cells (CTCs)

Circulating tumor cells (CTCs) are intact cancer cells that have shed from the primary tumor or metastatic sites and entered the peripheral blood, offering direct evidence of tumor invasion and dissemination. Unlike circulating tumor DNA (ctDNA), which provides fragmented genetic information, CTCs preserve the full cellular structure, allowing for both genomic and phenotypic profiling. Although rare in circulation, their clinical relevance is significant, as they provide valuable insights into prognosis, therapeutic targets, and metastatic risk. In colorectal cancer, for instance, higher abundance and phenotypic heterogeneity of CTCs, especially when accompanied by tumor-derived extracellular vesicles (tdEVs) in blood from tumor-draining veins, have been associated with adverse prognosis and higher likelihood of metastasis (Cieslik *et al.*, 2025; Yu *et al.*, 2022). Similarly, in rare cancers such as uveal melanoma and synovial sarcoma, the detection of CTCs has shown promise for monitoring disease progression and potential relapse (Fremder *et al.*, 2023; Hall *et al.*, 2021; Mihály *et al.*, 2018). However, challenges remain in their clinical application due to their low concentration in blood and significant variability in size and surface markers. Nevertheless, the capacity to scrutinize

intact viable cancer cells via CTC analysis is a robust technique to elucidate tumor behavior and for informing individualized treatment strategies.

2.3. Exosomes and Extracellular Vesicles (EVs)

Exosomes and other extracellular vesicles (EVs) are tiny, membrane-bound particles released by all types of cells, including cancer cells, into the bloodstream. These vesicles carry a rich mix of DNA, RNA, proteins, and lipids that mirror the biological state of their cells of origin, making them powerful messengers in cell-to-cell communication. In the context of cancer, especially colorectal cancer, tumor-derived EVs (tdEVs) have been found to display unique surface markers and protein signatures that correlate with disease stage and prognosis (Yu *et al.*, 2022). Their stability in circulation and their ability to reflect the tumor microenvironment make them highly valuable for non-invasive cancer detection through liquid biopsy. When analyzed alongside other biomarkers such as circulating tumor cells or cell-free DNA, exosomal content can significantly enhance the accuracy of diagnosis and monitoring. These vesicles are also involved in critical cancer processes like immune escape, metastasis, and resistance to therapy, which adds to their appeal as both diagnostic and prognostic tools. For instance, in pancreatic ductal adenocarcinoma, EVs have been used to differentiate between tumor and stromal elements in the blood, offering a clearer picture of tumor behavior and treatment response (Götze *et al.*, 2025; Glinge *et al.*, 2021). As research progresses, EVs are likely to become even more important in tracking disease progression and tailoring cancer therapy.

2.4. Cell-Free RNA (cfRNA) and MicroRNAs (miRNAs)

Cell-free RNA (cfRNA), including microRNAs (miRNAs), represents another class of promising biomarkers in liquid biopsy. Released by tumor cells into the circulation, cfRNA can reflect active gene expression changes occurring within the tumor. miRNAs, in particular, are short non-coding RNA molecules known for their stability in body fluids and their regulatory role in cancer pathways. Recent studies have demonstrated that miRNA profiles in plasma or urine can be highly indicative of cancer presence and subtype. For example, an integrated miRNA-SERS urine assay has been demonstrated to possess accurate performance for point-of-care diagnosing and molecular-stage classification of bladder cancer (Moisoiu *et al.*, 2022). 2. Likewise, a certain plasma miRNA signature has been explored for the early detection of oesophageal squamous cell carcinoma in both retrospective and prospective groups (Miyoshi *et al.*, 2022). These findings suggest that cfRNA and miRNAs are not only diagnostic tools but may also serve in monitoring therapeutic response and detecting recurrence.

2.5. Tumor-Educated Platelets (TEPs)

Tumor-educated platelets (TEPs) are a promising and emerging class of liquid biopsy biomarkers that result from the interaction between circulating platelets and tumor-derived factors. This interaction leads to notable changes in the RNA content and function of the platelets, effectively turning them into carriers of tumor-specific molecular signals. Because platelets are abundant, stable in circulation, and easy to isolate, they offer an accessible platform for non-invasive cancer detection. Their RNA profiles have been shown to undergo tumor-induced splicing changes, reflecting both local and systemic tumor activity. Recent findings suggest that TEP RNA signatures can be highly informative, capable of detecting minimal residual disease and predicting early recurrence in cancers such as gastric cancer (Li *et al.*, 2022). Although the study of TEPs is still in its early stages and they

have not been the primary focus of most liquid biopsy research to date, they are gaining attention as a sensitive and complementary tool to established biomarkers like circulating tumor DNA and circulating tumor cells, particularly in scenarios where tumor burden is low or traditional biopsy methods are limited.

3. Technologies Used in Liquid Biopsy

3.1. Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) is a powerful molecular tool widely applied in liquid biopsy for identifying genetic alterations in tumor-derived material from various body fluids such as blood, cerebrospinal fluid (CSF), urine, and saliva. Unlike traditional tissue biopsies, liquid biopsy is minimally invasive and allows for repeat sampling over time, making it ideal for monitoring disease progression and treatment response.

NGS enables the analysis of multiple circulating biomarkers including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), RNA, and exosomes. Of these, ctDNA is the most frequently used, as it reflects the tumor's genetic landscape and can reveal mutations, insertions and deletions, gene amplifications, copy number variations, structural rearrangements, and even epigenetic changes. The extracted DNA is amplified and sequenced using either targeted gene panels or broader approaches like whole-exome or whole-genome sequencing. This high-throughput technique offers exceptional sensitivity, with the ability to detect mutant allele frequencies as low as 0.01 percent, making it especially useful for early-stage cancer detection and post-treatment surveillance (Walls *et al.*, 2020). Clinically, NGS has proven valuable across a range of cancers. Walls *et al.* (2020) demonstrated that ultra-deep NGS could track early ctDNA changes during radiotherapy in patients with non-small cell lung cancer, offering insights into treatment efficacy. In colorectal cancer, Rubio-Mangas *et al.* (2023) introduced the Differential Presence of Exons (DPE) method, which utilized cfDNA sequencing to stratify patients more effectively than conventional mutation analysis. In the context of neuro-oncology, where tissue access is limited, NGS of CSF has emerged as a critical diagnostic aid. (Zhao *et al.* 2019) reported that ctDNA analysis from CSF via NGS improved diagnostic sensitivity in meningeal carcinomatosis compared to traditional cytology. Building on this, Navrkalova *et al.* (2025) confirmed that multi-biomarker NGS profiling can reliably detect CNS lymphoma-derived ctDNA in CSF, highlighting its diagnostic relevance in central nervous system tumors.

Despite these advantages, challenges remain. These include limited ctDNA shedding in some early-stage cancers, high costs, and the complexity of interpreting large volumes of sequencing data. Nevertheless, NGS is gaining traction in clinical practice, supported by U.S. FDA-approved platforms such as Guardant360 CDx for mutation detection, FoundationOne Liquid CDx for comprehensive genomic profiling, and Signatera for personalized monitoring of minimal residual disease (Rubio-Mangas *et al.*, 2023; Navrkalova *et al.*, 2025). These examples underscore NGS's growing role in advancing precision medicine through non-invasive cancer diagnostics.

3.2. Digital PCR (dPCR)

Digital PCR (dPCR) is a highly sensitive and quantitative technique used in liquid biopsy for detecting low-frequency genetic changes with great accuracy. The method works by dividing a DNA sample into thousands or millions of tiny individual reactions, where each ideally contains either none

or just one copy of the target DNA. PCR amplification then takes place separately in each partition. After amplification, the presence or absence of a fluorescent signal in each compartment is measured, and the absolute number of target molecules is calculated using Poisson statistics. This approach eliminates the need for standard curves or reference controls (Zmrzljak *et al.*, 2021). A commonly used form of this technology is droplet digital PCR (ddPCR), which utilizes microfluidic systems to encapsulate DNA into thousands of oil-based droplets, each serving as its own reaction chamber. This setup allows for extremely sensitive detection of rare mutations, making ddPCR particularly valuable in analyzing complex biological fluids like blood or vitreous fluid (Overs *et al.*, 2024).

In the setting of liquid biopsy, dPCR is most commonly used to detect ctDNA. It can also be employed to assess gene methylation, copy number variations, and, to a lesser extent, nucleic acids derived from CTCs and exosomes. The technique's sensitivity allows it to detect mutant allele frequencies as low as 0.01 percent, making it ideal for early cancer detection, monitoring minimal residual disease, and evaluating treatment response (Crucitta *et al.*, 2023). Multiple studies have validated its clinical use. Zmrzljak *et al.* (2021) successfully used ddPCR to detect KRAS and NRAS mutations in plasma samples from colorectal cancer patients. Overs *et al.* (2024) applied ddPCR to identify methylation markers such as COL25A1 and METAP1D, showing potential as epigenetic biomarkers for colorectal cancer. Nell *et al.* (2025) further demonstrated the method's adaptability by using ddPCR to analyze vitreous fluid for genetic profiling of uveal melanoma. These findings emphasize ddPCR's utility across different tumor types and sample sources.

Among its key strengths, dPCR offers absolute quantification, excellent sensitivity, reproducibility, relatively low cost, and a quick turnaround. However, its main limitation lies in its low multiplexing capability, which restricts the number of mutations that can be analyzed simultaneously. For broader mutation profiling, next-generation sequencing remains the preferred approach (Crucitta *et al.*, 2023).

Commercial systems such as the Bio-Rad QX200 and Thermo Fisher QuantStudio Absolute Q are commonly used in research and clinical labs. While most dPCR assays are offered as laboratory-developed tests within certified clinical settings, they are increasingly being integrated into precision oncology practices, especially for tracking known mutations during targeted therapies.

3.3. Quantitative real-time methylation and unmethylation Liquid Pcr

Detecting DNA methylation through liquid biopsy is quickly becoming one of the most promising tools for finding cancer early, sometimes even before symptoms appear. A key early change in many cancers is something called Hyper methylation, where certain DNA regions, especially the promoters of Tumor suppressor genes, become overly methylated. When this happens, those protective genes get switched off, which allows cancer to grow Unchecked. Liquid biopsy mainly looks at fragments of tumor DNA circulating in the bloodstream known as circulating tumor DNA (ctDNA) But it can also pick up signals from circulating tumor cells (CTCs) and Extracellular vesicles (EVs). Together, these sources can offer a more complete picture of what the tumor is doing (Keup *et al.*, 2021).

The process starts with a simple blood draw. From the blood, plasma is separated and used to extract what's called cell-free DNA (cfDNA). This DNA comes from both healthy and cancerous cells,

but in very small amounts Especially in early stages so highly sensitive methods are essential. To detect methylation, the DNA is first treated with a chemical called Bisulfite. This method works by converting unmethylated cytosines into Uracils, while the methylated ones remain unchanged, helping to tell the Difference between the two. That difference becomes important in the next step. To measure the methylation, scientists often use a method called Digital droplet PCR (ddPCR). In this technique, the sample is broken into nearly 20,000 tiny droplets, each acting like a mini test. Special fluorescent probes are added that glow in different colors depending on whether the DNA in a droplet is methylated or not, green (FAM) for methylated, and yellow (HEX) for unmethylated. These signals are then counted across all Droplets, giving an exact measure of how much methylation is present. This accuracy is incredibly useful, especially when the goal is to detect just a few Fragments of cancer DNA in the blood (Wang *et al.*, 2021). This kind of test offers some big advantages. It's non-invasive, very sensitive, and gives real-time information about what's happening in the body. That Makes it not only useful for early diagnosis, but also for tracking how well a Patient is responding to treatment, or spotting if the cancer comes back. Of Course, it's not perfect bisulfite treatment has to be done carefully to avoid Errors, and since most tests target only a few specific genes, they might Miss changes happening elsewhere in the genome. Also, in some slow Growing or early cancers, there may not be enough ctDNA in the blood to Detect. Still the progress is exciting. Tools like the QX200 ddPCR system From Bio-Rad are already widely used in research labs. And in the clinic, Tests like Epi proColon which screens for colorectal cancer by detecting Methylated SEPT9 in blood have already been approved by the FDA. As the Technology continues to evolve, DNA methylation analysis through liquid Biopsy is likely to play an even bigger role in personalized cancer care, helping doctors catch cancer sooner, tailor treatments more precisely, and Monitor recovery with just a simple blood test

3.4. Surface-enhanced Raman scattering (SERS)

Surface-enhanced Raman scattering (SERS) is a smart and sensitive technique that helps doctors detect cancer-related signals from simple body fluids like blood, serum, or urine without needing any surgery or complicated procedures. It works using extremely tiny gold or silver particles that act like magnifiers. These particles boost the weak signals that come from cancer-related molecules such as circulating tumor DNA(ctDNA), tumor cells (CTCs), small RNAs (miRNAs), or exosomes. When these molecules come close to the metal particles, they give off a special kind of signal called a "Raman signal," which becomes strong enough to detect. Scientists can even customize these particles to catch specific cancer molecules, making the test very accurate and focused. For example, in a 2024 study by Zhai and team, blood samples from lung cancer patients were tested using SERS and analyzed with the help of a computer model. The system could clearly tell who had lung cancer and who didn't, just from a small amount of blood, and without needing any dyes or labels. In another study by Lin *et al.* (2022), urine samples were used to detect bladder cancer. The team used SERS along with miRNA analysis to not only find cancer but also understand its type, helping guide future treatment. The whole process was quick, painless, and could even be done at the point of care.

What makes SERS special is its ability to catch cancer early even when the amount of cancer-related material in the body is extremely low. It's fast, works with very small sample sizes, and can test for many signals at the same time. Still, there are a few issues that need fixing. The tiny metal particles

need to be made exactly the same each time for the test to be reliable, and analyzing the results needs advanced software. So far, there are no FDA-approved SERS-based cancer tests, but scientists are working on making this technology ready for regular hospital use Zhai *et al.* (2024) and Lin *et al.* (2022), it's clear that SERS has great potential to become a powerful tool in the early detection and monitoring of cancer.

3.5. Next-generation sequencing (NGS) of the whole miRnome

Next-generation sequencing (NGS) of the whole miRnome is a smart and advanced method that helps researchers look at all the microRNAs (miRNAs) in a sample-like urine or blood. These miRNAs are tiny molecules that control how genes work, and changes in their levels can signal the presence of cancer. What makes NGS special is that it doesn't just look for a few miRNAs it scans all of them at once, giving a complete picture. Scientists begin by collecting RNA from the sample, turning it into a readable form, and running it through a sequencing machine that reads and counts each miRNA. This helps identify which miRNAs are present in higher or lower amounts in people with cancer compared to healthy individuals. In a study by Lin *et al.* (2022), this technique was combined with another advanced method called SERS (surface-enhanced Raman scattering) to create a new, non-invasive urine test for bladder cancer. First, they used NGS to find the specific miRNAs that were linked to bladder cancer. Once they knew which miRNAs to focus on, they used SERS to build a quick and sensitive test that could detect those markers directly in urine. This combined approach helped not only in diagnosing bladder cancer early but also in identifying its type, which is important for choosing the right treatment. What's amazing is that this test could be done quickly and easily, even in a clinic, without the need for any surgery or complex lab work.

The beauty of NGS is that it allows discovery; you don't need to know what you're looking for ahead of time. It can find known miRNAs and even new ones that might be linked to cancer. It can test many miRNAs in one go, giving a detailed view of what's happening in the body. However, it does require expensive machines and trained people to handle the data, which can make it hard to use in everyday hospitals. That's why combining NGS with something fast and simple like SERS is such a great idea. The study by Lin *et al.* shows how this mix of deep analysis and rapid testing could lead to practical, painless, and accurate cancer detection tools making it easier for doctors to catch diseases early.

4. Genomic and epigenomic features of cell free DNA

In recent years, liquid biopsy has emerged as a powerful alternative to traditional tissue biopsies, especially in cancer research. This method focuses on analyzing cell-free DNA (cfDNA) short fragments of DNA that circulate in the bloodstream. What makes cfDNA particularly valuable is that it carries both genetic (genomic) and regulatory (epigenomic) signatures of cancer, providing insights into the tumor's behavior, origin, and progression all from a simple blood sample.

- **Genomic Characteristics of cfDNA**

The genomic features of cfDNA refer to actual changes in the DNA sequence that are commonly associated with cancer. These include single nucleotide mutations, copy number variations, insertions, deletions, and even larger structural alterations such as chromosomal rearrangements and gene fusions. Tumor-derived cfDNA often mirrors the mutation profile found in the primary tumor. For

instance, frequent mutations are seen in cancer-driver genes like TP53, KRAS, and PIK3CA, which play major roles in cancer development and drug resistance (Bronkhorst *et al.*, 2021).

One unique genomic trait of cfDNA in cancer is its fragmentation pattern. cfDNA fragments are usually shorter than cfDNA from normal cells and display specific cleavage sites related to chromatin structure and nucleosome organization. This pattern is not random it reflects how DNA is packed inside cancer cells and how it is released during cell death (Cristiano *et al.*, 2019). By analyzing these genome-wide fragmentation patterns, researchers can not only detect the presence of cancer but also predict its tissue of origin and subtype. Moreover, these fragment size signatures have become so specific that they can distinguish between early and late-stage tumors, and even between different types of cancer, offering a broader and more sensitive approach than mutation detection alone.

- **Epigenomic Characteristics of cfDNA**

While mutations provide essential clues, epigenetic changes offer another layer of critical information. Epigenomic features refer to modifications that affect gene activity without altering the DNA sequence. One of the most widely studied epigenetic marks in cancer is DNA methylation, a process where methyl groups are added to cytosine bases, often leading to gene silencing. In cancer, hypermethylation of promoter regions of tumor suppressor genes like APC, CDKN2A, and RASSF1A is a common event. These abnormal methylation patterns are preserved in cfDNA and can be accurately detected using blood samples (Shen *et al.*, 2021).

Since methylation profiles are often tissue-specific, analyzing them in cfDNA can also help determine the tumor's tissue of origin, which is especially useful in cases of metastatic or unknown primary cancers. Beyond methylation, chromatin accessibility which describes how “open” or “closed” a DNA region is can also be inferred from cfDNA. In cancer cells, certain genomic regions become more active or repressed due to changes in chromatin structure. These shifts are reflected in the fragmentation and nucleosome patterns of cfDNA. For example, cfDNA tends to be cleaved at exposed regions of chromatin, which correspond to active genes and enhancers (Snyder *et al.*, 2016). A study by Sheng *et al.* (2021) in gastric cancer showed that the activity of gene enhancers regulatory elements that boost gene expression varied across tumors and was influenced by the binding of transcription factors. These differences in enhancer activity could also be detected through epigenomic profiling of cfDNA, offering insights into tumor heterogeneity and regulatory disruptions.

- **Combined Genomic and Epigenomic Insights**

What makes cfDNA analysis especially powerful is the ability to combine genomic and epigenomic data for a more complete understanding of cancer. While mutation analysis helps detect cancer-related genes, adding methylation patterns, chromatin accessibility, and nucleosome positioning allows for better sensitivity and specificity, particularly in early-stage cancers or minimal residual disease (Bronkhorst *et al.*, 2021). Integrative studies now use multiple layers of cfDNA information fragment size, mutation load, DNA methylation, and enhancer signals to identify cancers more accurately and even to predict how patients will respond to treatment. The work by Leger *et al.* (2022) also emphasized how genetic background influences epigenomic variability, reminding us that personalized interpretation of cfDNA features may enhance diagnostic precision.

5. Applications:

5.1. Tumor Genotyping and Targeted Therapy Selection

Liquid biopsy allows for the detection of specific genetic changes in tumors through a simple blood test. This technique is essential for identifying actionable mutations such as EGFR, ALK, and KRAS, which help oncologists select the most appropriate targeted treatments for patients especially when traditional tissue biopsies are challenging or unsafe to perform. It is particularly useful in managing non-small cell lung cancer (NSCLC), where rapid mutation detection can guide therapy with tyrosine kinase inhibitors. Because liquid biopsy is minimally invasive, it enables quicker and more comfortable genetic analysis, promoting personalized medicine (Smith *et al.*, 2024)¹.

5.2. Detection of Minimal Residual Disease (MRD) and Early Relapse

Liquid biopsy offers a powerful way to detect minimal residual disease—those few cancer cells that might still linger after surgery or treatment and could eventually cause the cancer to return. Standard imaging often doesn't catch these cells until the disease is already advancing (Smith *et al.*, 2024)¹. But with circulating tumor DNA (ctDNA) testing, doctors can pick up on these traces of cancer at a much earlier, molecular stage. This gives them a chance to step in sooner with additional treatment if needed. It's especially helpful in cancers like breast and colorectal cancer, where spotting a recurrence early can make a big difference in the patient's outcome.

5.3. Tumor Genotyping & Drug Selection

Liquid biopsy is changing the game when it comes to identifying treatable mutations in lung cancer, especially in the EGFR gene. In a study by Wei & Wu (2025), researchers used real-time PCR (qPCR) to analyze blood samples and detect common EGFR mutations, such as the exon 19 deletion and the L858R point mutation. These specific mutations are strong indicators that a patient may respond well to EGFR-targeted therapies like erlotinib, gefitinib, or afatinib. The real advantage here is that these genetic changes can be picked up directly from a blood sample—no need to wait for invasive tissue biopsies or long pathology reports. This approach is especially helpful in cases where tumor tissue isn't available, can't be safely accessed, or when a biopsy would pose too much risk. In short, liquid biopsy provides a faster, safer, and highly effective path to personalized cancer treatment (Wei & Wu, 2025).

5.4. Early Detection, Screening, and Diagnosis of Cancer

Liquid biopsy represents a promising, non-invasive approach for the early identification of malignancies by analyzing circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), circulating tumor cells (CTCs), and tumor-derived extracellular vesicles. This technique allows for repeated sampling with minimal patient discomfort, offering the potential to detect cancers earlier than traditional imaging or tissue biopsies. According to recent findings by Satpathy *et al.* (2025), liquid biopsy holds significant promise for cancer screening programs, particularly in high-risk populations. It may facilitate the detection of asymptomatic tumors and help reduce delays in diagnosis. Furthermore, this method can serve as a valuable complement to imaging, especially when tumors are located in regions that are difficult to access surgically.

5.5. Subtype-Specific Detection and Monitoring in Lymphomas

In hematologic malignancies particularly lymphomas, liquid biopsy has demonstrated substantial clinical value. The presence of ctDNA mutations can aid in both the accurate classification of lymphoma subtypes and ongoing disease monitoring. For example, in conditions such as follicular lymphoma and diffuse large B-cell lymphoma, customized ctDNA assays have proven capable of detecting minimal residual disease (MRD) and identifying early molecular relapse, often before changes appear in imaging or standard lab tests (García-Silva *et al.*, 2024). This is especially relevant for patients undergoing advanced therapies like CAR-T cell treatment, where real-time molecular surveillance enhances treatment decision-making by offering high sensitivity and specificity.

5.6. Prognostic Significance

Measurement of ctDNA levels in cancer patients' plasma offers valuable prognostic information across various tumor types. In colorectal cancer, the presence of ctDNA following surgery is strongly linked to an increased risk of recurrence and often precedes radiologic signs of disease return (Satpathy *et al.*, 2025). Similarly, in non-small cell lung cancer (NSCLC), the appearance of resistance mutations such as EGFR T790M in ctDNA has been associated with shorter progression-free survival. These molecular changes can serve as early indicators of treatment resistance, underlining the role of liquid biopsy as a biomarker for both minimal residual disease and therapeutic efficacy.

5.7. Therapeutic Decision-Making and Personalized Treatment

Liquid biopsy is increasingly used to support personalized oncology by detecting actionable genetic mutations that guide treatment choices. In NSCLC, for instance, the identification of EGFR mutations in plasma samples enables the initiation of targeted therapies without the need for invasive tissue biopsies (Amrita *et al.*, 2025). Moreover, liquid biopsies facilitate ongoing evaluation of therapeutic effectiveness and can detect resistance mutations early, allowing for timely modifications to treatment strategies. This approach supports patient stratification based on molecular risk and enables the dynamic adjustment of treatment intensity, enhancing the precision of cancer management. Serial liquid biopsy assessments—postoperatively or during systemic therapy—offer real-time insights that improve clinical outcomes.

5.8. Expanding Applications Beyond Cancer

The utility of liquid biopsy is also being explored in several non-oncologic fields, including neurodegenerative, cardiovascular, infectious, and autoimmune diseases. Biomarkers extracted from blood or cerebrospinal fluid—such as cfDNA, RNA, and exosomal proteins—show promise for early diagnosis and disease tracking in conditions like Alzheimer's disease, Parkinson's disease, and systemic lupus erythematosus (Satpathy *et al.*, 2025). In cardiology, cfDNA derived from damaged heart tissue is under investigation as a potential marker for myocardial injury and the progression of heart failure. These emerging applications highlight the broad clinical relevance of liquid biopsy across diverse areas of medicine.

6. Advantages

6.1 Improved Sensitivity and Detection Techniques

To pick up tiny amounts of circulating tumor DNA (ctDNA), especially in early cancers or when only a small amount of disease remains, ultra-sensitive sequencing technologies have been

developed. Methods like CAPP-Seq combined with digital error correction can detect mutations present at incredibly low levels less than 0.01%. Personalized tests that focus on mutations unique to each patient's tumor, identified from their tissue samples, have further boosted accuracy and clinical usefulness (Satpathy *et al.*, 2025; Coppola *et al.*, 2025). On top of this, analyzing the size and epigenetic patterns of cfDNA adds extra detail, helping doctors detect cancer earlier and with more confidence (GI Cancer Review, 2025).

6.2 Standardizing Sample Handling and Testing

Global efforts are underway to standardize how blood samples are collected, processed, and stored to protect the quality of cfDNA. Following these agreed-upon procedures helps labs get consistent results, making it easier to compare data from different testing platforms and ensuring reliable clinical use (Coppola *et al.*, 2025; Satpathy *et al.*, 2025).

6.3 Combining Multiple Data Types and Smarter Analysis

Because cfDNA data is complex, researchers now combine multiple types of molecular information such as mutation detection, DNA methylation patterns, and fragment size to better identify cancer-specific signals. Advanced bioinformatics tools also help distinguish true tumor mutations from background noise like clonal hematopoiesis, improving the precision of liquid biopsy tests (Coppola *et al.*, 2025).

6.4 Exploring Other Body Fluids and Circulating Tumor Cells

Liquid biopsy isn't limited to blood. Researchers are exploring other fluids like bile, urine, and gastric juice, especially for GI cancers where tumor DNA might be more concentrated locally (GI Cancer Review, 2025). At the same time, new microfluidic and automated technologies are making it easier to capture and study circulating tumor cells (CTCs). These cells provide extra biological information that complements cfDNA analysis and can be used for further functional testing (Satpathy *et al.*, 2025).

6.5 Bringing Liquid Biopsy into Clinical Practice

National health programs like those led by the UK's NHS are starting to use liquid biopsy as a first-line diagnostic tool for cancers such as lung and breast cancer. This integration helps reduce the time to diagnosis and increases patient access, highlighting the growing importance of liquid biopsy in everyday medical care (Satpathy *et al.*, 2025).

6.6 Using Artificial Intelligence to Interpret Data

Because liquid biopsy generates a huge amount of complex data, AI-based tools are being developed to assist clinicians. These systems analyze multiple molecular signals and turn them into clear, actionable insights, helping doctors make faster and more accurate treatment decisions (Coppola *et al.*, 2025).

6.7 Rapid Turnaround Time

Traditional liquid biopsy workflows can take several hours to days, potentially delaying clinical decision-making. Herzog *et al.* (2025) introduced a fully automated single-cell CTC platform that streamlines sample processing, cell isolation, and RNA profiling within approximately one hour. This rapid turnaround enables near real-time molecular diagnostics, supporting timely therapeutic interventions and improving patient management.

6.8 Minimally Invasive and Repeatable Sampling

Liquid biopsy, which typically relies on peripheral blood or other bodily fluids, offers a less invasive alternative to conventional surgical tissue biopsies. This reduces patient discomfort and procedural risks while permitting serial sampling over time. Such longitudinal monitoring allows for the dynamic assessment of tumor progression, treatment response, and early relapse detection (Mondal *et al.*, 2024; Revelo *et al.*, 2019). The capacity for repeated sampling enhances clinical flexibility and facilitates personalized treatment strategies.

6.9 Comprehensive Multi-Omic Profiling

Modern liquid biopsy platforms enable simultaneous interrogation of multiple biomarker classes—including ctDNA mutations, CTC RNA expression profiles, and epigenetic modifications from a single specimen (Herzog *et al.*, 2025). This integrative, multi-omic approach provides a more comprehensive tumor profile that reflects both genetic diversity and phenotypic heterogeneity, which is crucial for understanding mechanisms of therapeutic resistance and identifying actionable targets.

Applicability to Hard-to-Biopsy Tumors

Certain malignancies, such as glioblastoma, pose significant challenges for tissue biopsy due to their location and associated risks. Bauman *et al.* (2022) reported that liquid biopsy approaches utilizing cerebrospinal fluid-derived cell-free DNA and exosomes can overcome these limitations, offering a non-invasive method for molecular profiling of brain tumors. This expands the scope of liquid biopsy beyond traditionally accessible cancers and opens new avenues for managing central nervous system malignancies.

7. Limitations:

1. Biological Limitations

Low ctDNA Levels in Early-Stage or Low-Tumor Burden Cases

One of the biggest biological hurdles with liquid biopsy is the low amount of circulating tumor DNA (ctDNA) in patients with early-stage cancers or minimal tumor burden. Particularly in gastrointestinal (GI) and solid tumors like colorectal and gastric cancers, tumors may release ctDNA in amounts too small for even the most sensitive tests to detect (Satpathy *et al.*, 2025; Coppola *et al.*, 2025). As a result, these cases are at risk for false-negative results, especially when monitoring for minimal residual disease (MRD).

Tumor Heterogeneity and Uneven DNA Shedding

Tumors don't shed DNA uniformly. Even though liquid biopsy is meant to pick up DNA from across the body, inconsistent shedding especially from GI tumors or liver metastases can lead to incomplete or skewed genetic profiles. Some mutations or resistant tumor clones might go undetected simply because they're not shedding DNA into the bloodstream in measurable amounts (GI Cancer Review, 2025).

2. Technical Limitations

Variability in Sample Handling and Pre-Analytical Steps: From the type of collection tube used to how quickly the sample is processed and whether plasma or serum is tested, small differences in handling can significantly affect the quality and quantity of cell-free DNA (cfDNA). These

inconsistencies create challenges in ensuring reliable results across different labs or studies (Coppola *et al.*, 2025).

Lack of Standardization in Testing Platforms: Liquid biopsy is not a one-size-fits-all technology. With various testing methods like ddPCR, NGS, CAPP-seq, and BEAMing available each with different strengths there's no universally accepted standard. This makes it hard to compare results between labs or healthcare centers, limiting its reliability in broader clinical use (Satpathy *et al.*, 2025).

Challenges with Non-Blood Samples: Fluids like bile, gastric juice, or urine can sometimes provide a better tumor signal, especially for certain GI cancers. However, these samples are harder to collect and don't yet have standardized testing procedures, which makes them less practical for routine use (GI Cancer Review, 2025; Coppola *et al.*, 2025).

3. Interpretation and Bioinformatics Challenges

False Positives from Non-Cancer Sources

Not all detected mutations come from tumors. In many cases, mutations related to aging like those found in clonal hematopoiesis (CHIP) can show up in the results. These mutations (commonly in genes like TP53, DNMT3A, and KRAS) may be mistaken for cancer-related mutations, which could lead to unnecessary treatments or anxiety (Coppola *et al.*, 2025; Satpathy *et al.*, 2025).

Tumor-Informed Testing Is More Accurate—but Not Always Feasible

Generic, or “tumor-agnostic,” tests are quicker but more prone to picking up irrelevant background mutations. More accurate “tumor-informed” approaches rely on having access to the patient's tumor tissue for comparison, which adds time, cost, and complexity (Satpathy *et al.*, 2025).

High Computational Demands

Analyzing the complex data from high-throughput sequencing of cfDNA isn't easy. It requires powerful computing tools, advanced algorithms, and specialized personnel. These requirements drive up costs and create access barriers, particularly in low-resource or rural settings (Coppola *et al.*, 2025).

4. Clinical and Regulatory Challenges

Limited Clinical Evidence Across Cancer Types

Although liquid biopsy is widely used in cancers like non-small cell lung cancer (NSCLC) and colorectal cancer (CRC), many other GI cancers lack strong clinical validation especially in early-stage cases. More large-scale, prospective studies are needed to prove its effectiveness in a broader range of diseases (GI Cancer Review, 2025).

High Cost and Limited Availability

The advanced technologies and personalized assays required for liquid biopsy can be prohibitively expensive. This limits access for patients in developing countries and even in smaller clinics or hospitals outside of major academic centers (Satpathy *et al.*, 2025).

Reimbursement and Ethical Issues

There are still gaps in insurance coverage for liquid biopsy, making it financially inaccessible for many patients. On top of that, there's growing concern around how to handle unexpected findings like inherited genetic risks or non-cancer DNA (e.g., CHIP, fetal DNA) which raise ethical and legal questions without clear guidelines (Satpathy *et al.*, 2025).

8. The Future of Liquid Biopsy: A New Era in Cancer Care

Liquid biopsy is moving from being just a helpful diagnostic tool to becoming a central part of personalized cancer care. With ongoing advances in science, technology, and clinical practice, the future of this technique looks incredibly promising. Researchers are now focusing on building a more complete picture of cancer by analyzing not just ctDNA, but also circulating tumor cells (CTCs), extracellular vesicles, RNA, and even epigenetic markers offering new insights into how tumors grow, change, and respond to treatment (Satpathy *et al.*, 2025; Coppola *et al.*, 2025).

1. Expanding Use in Cancer Screening and Early Detection

Looking ahead, liquid biopsy is expected to play a major role in cancer screening, especially for hard-to-detect cancers like those of the lung, pancreas, and colon. New tests that combine genetic mutations, DNA methylation, and fragment patterns are being developed to catch cancer in its earliest stages before symptoms even appear (Satpathy *et al.*, 2025). These advances could lead to earlier diagnoses and better outcomes. There's also growing interest in pairing liquid biopsy with imaging tests or risk assessment tools to make screening even more accurate (Coppola *et al.*, 2025).

2. Real-Time Monitoring and Catching Relapse Early

One of the biggest benefits of liquid biopsy is its ability to track cancer in real time. Because it only requires a blood sample, doctors can monitor patients more often during and after treatment. This is especially useful for detecting minimal residual disease (MRD) the tiny traces of cancer that might be left behind after surgery or chemotherapy. Personalized MRD tests, based on a patient's specific tumor DNA, are already showing strong potential in breast and colorectal cancers. Soon, these tests may guide decisions about follow-up treatment, help catch relapses early, and reduce unnecessary therapies (GI Cancer Review, 2025; Satpathy *et al.*, 2025).

3. Matching Patients to the Right Treatments

Liquid biopsy also opens the door to more personalized treatment. By analyzing how a tumor's DNA changes over time especially when resistance mutations start to appear doctors can quickly switch to a more effective drug. In the near future, it's likely that many treatment decisions will be guided by these blood-based tests instead of repeat biopsies. This not only saves time but also avoids the risks and discomfort of surgical procedures (Coppola *et al.*, 2025).

4. Tapping Into Other Body Fluids for Better Accuracy

While blood is the most common fluid used for liquid biopsy, researchers are finding that other fluids like bile, urine, saliva, cerebrospinal fluid, and even fluid around the lungs—may carry higher concentrations of tumor DNA in certain cancers. For example, bile-based tests are being explored for liver and pancreatic cancers, and spinal fluid is becoming important in brain tumors. These samples might offer more accurate results in cases where blood tests fall short, though more work is needed to standardize how these samples are collected and processed (GI Cancer Review, 2025).

5. Getting More Out of Circulating Tumor Cells (CTCs)

Although most liquid biopsy research focuses on ctDNA, circulating tumor cells offer a unique advantage: they provide a snapshot of how the tumor behaves. New tools are making it easier to capture these rare cells, analyze them at the single-cell level, and even test how they respond to drugs in the lab.

In the future, this could help doctors choose therapies based not just on the tumor's genetics but also on how the actual cells respond to treatment (Satpathy *et al.*, 2025).

6. Making Sense of Complex Data with Smart Tools

With so much data coming from these tests—mutations, methylation, cell profiles—it's becoming harder for doctors to interpret everything manually. That's where advanced software and decision-support tools come in. These systems bring all the pieces together and offer clear, actionable guidance to help clinicians make informed decisions faster. They'll also help flag treatment resistance earlier and even automate parts of the lab workflow, making testing more efficient and widely available (Coppola *et al.*, 2025).

7. Moving Toward Global Adoption

Several countries are already testing liquid biopsy in national health programs. For instance, the UK's NHS has begun using it for lung and breast cancer screening, leading to faster diagnoses and better access to care (Satpathy *et al.*, 2025). As the evidence continues to grow and the cost of testing comes down, more healthcare systems are expected to follow suit. Eventually, liquid biopsy could become a regular part of cancer care around the world especially as regulatory agencies approve broader use and integrate it into official treatment guidelines alongside imaging and digital pathology.

9. Recent Developments in Liquid Biopsy

Liquid biopsy technologies have seen rapid advancements, resulting in more sensitive and specific cancer detection and monitoring methods. A significant advancement was made by Herzog *et al.* (2025), who developed a fully automated platform for isolating single circulating tumor cells (CTCs). This innovation allows for quick and high-purity isolation, along with RNA profiling of individual CTCs. The platform stands out by enabling the first liquid biopsy-based expression profiling of antibody–drug conjugate (ADC) targets, including HER2, TROP2, Nectin-4, and c-MET, directly from patient blood samples. Compared to traditional methods that take six hours or more, this system achieves a 10–100-fold increase in CTC purity and completes the entire process within just one hour. Additionally, the platform maintains ctDNA integrity, enabling simultaneous multi-omic analysis from a single blood sample, thereby improving clinical decision-making by combining genomic and transcriptomic insights.

Building on this, Satpathy and colleagues (2025) reviewed the latest advances in molecular marker detection techniques, highlighting how tools like droplet digital PCR (ddPCR), BEAMing, and CAPP-Seq are becoming key players for detecting circulating tumor DNA (ctDNA) with great sensitivity. They also emphasized the exciting rise of single-cell RNA sequencing and DNA methylation profiling, which are transforming how we detect cancer—especially early-stage tumors and minimal residual disease (MRD). These technologies offer a much clearer picture of tumor diversity and how it changes during treatment, which is crucial for tailoring therapies to each patient.

Focusing on gastrointestinal cancers, Mondal *et al.* (2024) showed that profiling ctDNA mutations through liquid biopsies can catch relapse and treatment resistance early, which greatly improves patient outcomes. Their work supports the idea of using repeated liquid biopsy tests to monitor cancer in real-time and adjust treatments for colorectal and pancreatic cancers accordingly.

In glioblastoma, Bauman and team (2022) made strides by using cerebrospinal fluid (CSF)-derived cell-free DNA and exosomal RNA to bypass the challenge of the blood-brain barrier. This non-invasive approach allows doctors to profile brain tumors molecularly and make personalized treatment decisions, which is especially valuable since traditional tissue biopsies are difficult in this disease.

Lastly, Revelo *et al.* (2019) set the stage in lung cancer by establishing ctDNA-based detection of EGFR mutations and resistance variants as a practical clinical tool.

Conclusion:

Liquid biopsy is slowly reshaping the way we diagnose and monitor cancer. Unlike conventional tissue biopsies that require surgery or invasive procedures, liquid biopsy uses simple body fluids like blood or urine to gather information about the tumor. It focuses on detecting various cancer-related components such as ctDNA, CTCs, EVs, miRNAs, and TEPs. These markers can offer critical insights into the tumor's current status, even at an early stage or after treatment.

One of the key strengths of this method is that it allows repeated testing over time without causing discomfort to the patient. This means doctors can track how well the treatment is working or if the cancer is starting to come back, all in real-time. Especially when using modern techniques like ddPCR and NGS, even small changes in the tumor's genetic or epigenetic structure—like DNA methylation—can be detected with great accuracy.

Still, the approach is not without challenges. Early-stage tumors might release very low amounts of biomarkers, making detection harder. Moreover, the lack of standard testing protocols, high costs, and difficulty in interpreting complex data can create roadblocks for routine use in clinics. However, with ongoing research and rapid technological progress, many of these issues are already being addressed.

Looking ahead, liquid biopsy has the potential to become a standard tool in personalized cancer care. As it becomes more accurate and accessible, it will likely be used not only for early detection but also for deciding treatment strategies based on a person's unique tumor profile. This could lead to better outcomes, fewer side effects, and more informed clinical decisions in the fight against cancer.

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